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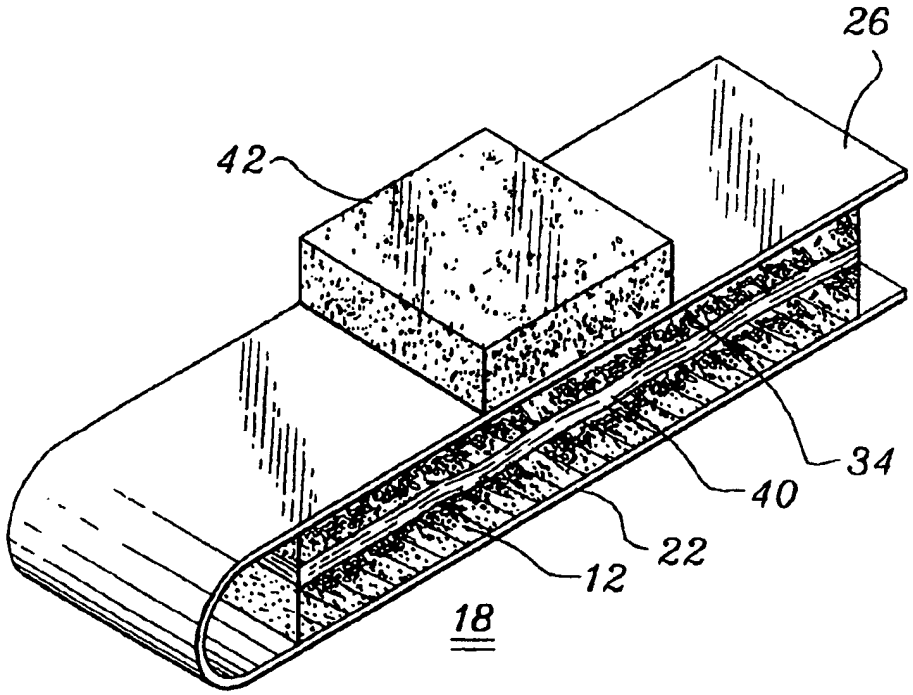


(57) Abstract

A dry chemistry reagent system for the detection of an analyte in a heterogeneous fluid sample includes a pad (42) for preconditioning of the fluid sample supported on upper surface (26), and essentially planar wicking element (34) for facilitating transport and uniform spreading of the sample fluid, an essentially planar porous membrane (12) having a porosity gradient from one planar surface thereof to the other supported on lower surface (22), and an impermeable barrier (40) between said wicking element (34) and said porous membrane (12).

DRY REAGENT THREE ELEMENT ANALYTE DETECTION SYSTEM

BACKGROUND OF THE INVENTION

Field of the Invention - This invention is directed to articles of manufacture and to analytical methods. More specifically, this invention resides in the provision of a highly effective delivery system for dry chemistry reagents, and in the incorporation of this delivery system into a plurality of diverse test strips. It is understood that the phrase "dry chemistry reagents" is inclusive of reagent systems for both clinical chemistry assays and immunoassays. This dry chemistry reagent system is useful in the rapid and efficient analysis of biological fluids. One of the unique features of this system is its ability to accomplish such analysis of samples containing cellular and particulate matter or macromolecules without prior separation of such endogenous materials from the sample. Such separation is generally desirable, and can be essential in certain analysis to prevent such material from masking detection of a reporter molecule which is indicative of the presence of the analyte of interest.

Description of the Prior Art - The search for a simple and effective means for performance of analytical testing of heterogeneous fluid samples, notably biological samples, has spanned more than twenty-five years. Much of the early work in this area was directed to the development of a reagent format which would be compatible with a simple yet effective analytical protocol. Probably one of the simpler of these systems was based upon the provision of a "dry reagent chemistry system", that is, a reagent system that was imbibed into an absorbent medium, dried and reconstituted by the simple addition/absorption of the fluid sample by the absorbent medium.

The patent and technical literature is voluminous regarding the development and refinement of such dry chemistry reagent systems. These systems have traditionally included the reagent coated tube technology, the reagent coated bead technology and

the so-called paper or bibulous layered systems. A format of dry chemistry reagent system which continues to enjoy increasing popularity is based upon single and multi-layered bibulous media.

One of the earlier and more successful of adaptations of the bibulous media format for dry chemistry reagent systems involved the development of a series of assays for analysis of whole blood. Some of the earlier patents in this field described the adaptation of this format to analysis of heterogeneous fluids (whole blood) for glucose and other common analytes (i.e., urea and cholesterol) of interest. The following list is representative of the patent literature directed to simple analytical devices (test strips) illustrating the adaptations of dry chemistry reagent system to analysis of heterogeneous biological fluids: U.S. Patents 3,061,523 (to Free); 3,552,925 (To Fetter); 3,607,093 (to Stone); 3,092,465 (to Adams); 3,298,789 (to Mast); and 3,630,957 (to Rey). Mast, Stone and other pertinent prior art are discussed below.

U.S. Patent 3,298,789 (to Mast) describes a test article for detection of glucose in heterogeneous fluid samples (i.e. whole blood). This test article is composed of a layer of bibulous material impregnated with a dry chemistry reagent system. This layer is overcoated with a smooth semi-permeable film of transparent ethyl cellulose. The test device is used to detect glucose in whole blood by simply applying a whole blood sample on the surface of the semi-permeable film coating which has been applied to the reagent impregnated material. After a brief interval, the fluid fraction of the sample is absorbed by the bibulous material. The cellular (colored) fraction of the sample is then wiped off the surface of the semi-permeable film to allow for observation/measurement of the indicator produced by reaction of the glucose and reagents within the bibulous layer. The surface characteristics of this semi-permeable film are thus critical to the operation of this device in the analytical environment. More specifically, the degree of smoothness of the protective film

(surface porosity) is critical and must be sufficiently fine to avoid penetration of the cellular components and hemoglobin fractions into the surface of the film. In most articles of this type the removal of cells and colored debris from the sample receptive surface of the test element can introduce analytical error into such analysis.

U.S. Patent 3,607,093 (to Stone) describes a liquid permeable membrane, of uniform chemical composition having, within its matrix, a dry chemistry reagent system. The membrane selected by Stone for his device is similar in its surface characteristics to the protective film of Mast. The analytical protocol utilizing the Stone device is also similar to that of Mast and requires the physical wiping of the sample receptive surface of the membrane for removal of cellular debris and colored materials (from the sample) to allow for observation/measurement of a reaction product indicative of the presence of the analyte of interest. The Stone analytical system suffers from the same deficiency which is common to Mast, namely, the introduction of analytical error by required physical removal or washing of the cellular and colored debris from the sample receptive surface of the analytical element prior to monitoring for indicator development.

Additional modification and enhancements have been made to the basic dry reagent chemistry formats described above. These modifications and enhancements have focused upon providing multiple test zones on a common test element; increasing the precision and correlation of indicator development with concentrations of analyte; and greater control in absorption/distributions of the sample within the bibulous reagent impregnated medium. The following patents are illustrative of such enhancements and modifications to the dry reagent chemistry format: U.S. Patent 3,847,822 (to Shuey); 3,802,842 (to Lange); 3,964,871 (to Hochstrasser); and 4,160,008 (to Fenochetti), and are discussed in further detail below.

U.S. Patent 3,802,842 (to Lange et al) describes a test strip incorporating a dry chemistry reagent system in which a sample receptive surface of an indicator (reagent) layer is covered by a fine mesh. The indicator layer can be supported upon a "colorless" or "transparent" support. The addition of the fine mesh to this test element reportedly results in enhancement in speed and uniformity of distribution of sample upon the surface of the indicator layer. This uniformity of distribution also reportedly results in substantial improvement in reproducibility of result.

U.S. Patent 3,847,822 (to Shuey) describes what he terms an "asymmetric" membrane comprising a polymer blend of polyvinyl pyrolidone and cellulose acetate. The composition of the membrane reportedly has improved transport of blood solutes, notable insulin, while being substantially impermeable to albumin.

U.S. Patent 3,964,871 (to Hochstrasser) describes a disposable indicator (test strip) having a built-in color intensity scale which is directly correlated to analyte concentration with a test sample. Accordingly, it is reportedly possible to simply immerse this indicator within a test sample and observe the progressive development in color within the various regions of the device. The development of color (indicator) within a specific region of the test strip can thus be directly correlated with a specific concentration of analyte.

U.S. Patent 4,160,008 (to Fenochetti) describes a test device for performance of a clinical analysis of a sample for different analytes on a common support. The inventor has isolated each distinct analytical test in separate zones on a common support by elevating the reagent specific layer above the common support and providing a blotter on the support to insure against run-off and cross-contamination of one analytical site by another.

The next generation (at least in terms of physical complexity) of dry chemistry reagent systems which has evolved is a multiple layered element having at least three discrete functional layers. These discrete functional layers are a spreading layer, a reagent layer and a signal (indicator) layer. Systems of this type used in the performance of glucose determinations on whole blood are the subject of numerous publications and issued patents. The following list is representative of the technical publications in this area: Walter, B., "Dry Reagent Chemistries in Clinical Analysis", Analytical Chemistry, Vol. 55, No. 4, pp. 498-514 (April, 1983); Curme, Henry G., et al., "Multilayer Film Elements for Clinical Analysis: General Concepts", Clinical Chemistry, Vol. 24, No. 8, pp. 1335-1342 (August, 1978); Ohkubo, Akiyuki, et al., "Plasma Glucose Concentrations of Whole Blood, as Determined with a Multi-layer-Film Analytical Element", Clinical Chemistry, Vol. 27, No. 7, pp. 1287-1290 (July, 1981); Ohkubo, Akiyuki, et al., "Multilayer-Film Analysis for Urea Nitrogen in Blood, Serum, or Plasma," Clinical Chemistry, Vol. 30, No. 7, pp. 1222-1225 (July, 1984); and Rupchock, Patricia, et al., "Dry-Reagent Strips Used for Determination of Theophylline in Serum," Clinical Chemistry, Vol. 31, No. 5, pp. 737-740 (May 1985). The following list is representative of the corresponding patent literature in this area: No. 4,042,335 (to Clement); No. 4,059,405 (to Sodickson, et al); No. 4,144,306 (to Figueras); No. 4,258,001 (to Pierce); and No. 4,366,21 (to Tom, et al); and No. 4,446,232 (to Liotta), Figueras and Liotta are more fully discussed below.

U.S. Patent 3,144,306 (to Figueras), describes a multi-layered analytical system in which an interaction of an analyte and non-diffusible reagents in the reagent layer, results in the release of a "performed detectable species" which can migrate from the reagent layer into a registration layer. This performed detectable species is then observed or measured in the registration layer. Figueras contemplates the adaptation of his system to glucose analysis of whole blood. The

introduction of the whole blood sample into the reagent layer of the Figueras element results in the release of a diffusible performed photographic dye, which is then free to migrate into the registration layer. Figueras requires the presence of an optical screen (radiation blocking layer) between the reagent layer and the registration layer to avoid masking or interference in detection of the dye from the non-diffusible color components (i.e., sample and reagents) in the reagent layer. Because of the requirement of maintenance of fluid contact between the various elements of the Figueras composite, its mechanical properties are critical. Accordingly, the multi-layered system of Figueras requires a supporting (transparent) layer to lend physical integrity to the device.

U.S. Patent 4,446,232 (to Liotta) describes a multiple layer test device for an immunoassay. This device and test protocol are directly analogous to that of the Figueras patent. The addition of a sample containing an analyte of interest results in the displacement of a previously immobile species. In the system described by Liotta, the component of the reagent system which is displaced is an enzyme conjugated to a member of an immunological binding pair. The conjugate is then carried into the laminate where it can effect production of a detectable species.

The immunochemical device and technique of the type described by Liotta is not readily compatible with whole blood analysis. It is possible to wash the test element for removal of the cellular debris, however, the effect of such an additional step upon the immunochemical binding process is not known and can be expected to introduce analytical error in such analysis. Manipulations of the test element can also be expected to mask detection of the analyte manifesting reaction or removal (in part) of the indicator species which is indicative of the analyte of interest. In addition, physical removal of cellular and colored debris will necessarily expose the clinician or the person performing the test to potential infection by those components of the blood which are removed from the test element.

As is evident for the foregoing discussion of the references appropriate for whole blood analysis, each type of test element generally requires a plurality of lamina in its preferred configuration. Where a single layer (component) test device is suggested, none of the references, with only one exception (U.S. 3,607,093 to Stone), either acknowledge or appreciate the potential chemical and optical interference of the erythrocyte population (and other colored components of the blood), on the analytical protocol or on the detection of the reaction product which is indicative of the presence of the analyte of interest. Where immobilization techniques are employed, the specificity of the binding reaction can, under certain conditions, be indiscriminate.

U.S. Patent No. 4,477,575 (to Vogel) and Vogel's improvement thereof, which is U.S. Patent No. 4,816,224, relate to a process, composition and device for the separation of plasma or blood serum from whole blood. Vogel, more particularly, teaches a method and means for the separation of plasma from whole blood and accomplishing a certain diagnostic analysis thereof through the passage of the whole blood through a matrix of glass fibers whereby plasma or serum is separated from the other components of the whole blood and is made available at both sides of a test strip matrix made in accordance with particular parameters of geometry and density of the glass fibers of which the matrix is formed. Further, the principles of operation Vogel employs the affinity of glass fibers to red blood cells. Also, the direction of flow of the test fluid is co-linear with the axes of the utilized glass fibers. In distinction, the instant invention employs a fiber orientation in the wicking layer thereof which is normal to the direction of fluid flow. Accordingly the fibers of the inventive wicking layer are co-parallel to the plane of the wicking layer. A further distinction between the respective systems is that, in the inventive system, contact between the layers thereof is a function of pressure or squeezing not osmosis as in Vogel.

U.S. Patent 4,632,901 (to Valkirs et al) teaches a method and apparatus for immunoassays. which is a multi-layer system particularly adapted for the detection of target antigens within a liquid sample. A first layer of the system constitutes a porous membrane or filter to which is bound an antibody of the target antigen to accomplish binding of the antigen to such first member. An osmotic output surface of the first member of Valkirs contacts an input layer to a second member thereof which second member comprises a body of absorbent material having osmotic properties in which the second layer detect antigens bound to said antibodies within the first member.

U.S. Patent No. 4,637,938 (to Dappen) teaches an assay for analysis of whole blood. The use of a multi-zone or multi-layer system includes a registration zone and a reagent/spreading zone which is provided with a void volume within the matrix thereof and an average pore size effective to accommodate whole blood. The agent within the reagent zone generates a reaction which can be spectrophotometrically detected at wavelengths greater than 600 nanometers.

U.S. Patent No. 4,774,192 (to Terminiello and Aronowitz (the within inventor)) teaches a dry chemistry reagent delivery system including a membrane having a porosity gradient therein. The present invention integrates a membrane of the type taught by Terminiello et al into an inventive multi-zone/multi-layer system having enhanced capability for the detection of analytes not readily or accurately detectable by the system of Terminiello et al.

U.S. Patent No. 4,790,979 (also to Terminiello and Aronowitz (the within inventor)) also teaches the use of a porous membrane impregnated with a dry chemistry reagent system specific for analysis of an analyte within whole blood and, in addition, teaches the use, in selectable cooperation with said porous membrane, of

a wicking element for the reception of a whole blood sample and the transport of such sample to the reagent system of the porous membrane. The system patent to Terminiello et al is of value primarily in the testing of whole blood samples for glucose and other analytes, but has proven to have little effectiveness with regard to a whole blood test which would be applicable to high density lipoprotein cholesterol, certain antigens, and proteins and enzymes associated with pregnancy.

The instant invention accordingly builds upon aspects of all of the above set forth prior art to thereby engender a dry chemistry reagent system for use under non-laboratory conditions and in connection with a range of different analyte tests not achievable in the prior art.

SUMMARY OF THE INVENTION

The instant invention relates to a dry chemistry reagent system for the detection of an analyte in a heterogeneous fluid sample. The system is, more particularly, a multi-layer system including a pad for pre-conditioning of the fluid sample, the pad comprising a porous compressible sponge-like material. Said pad comprises means for receipt of the fluid sample, said pad having an upper and lower surface. The system further includes an essentially planar wicking element having an upper and lower surface, said upper surface in proximal, non-contacting relationship to said lower surface of said pad, said wicking element comprising means for facilitating transport and uniform spreading of the fluid sample. The system further includes an essentially planar porous membrane having a porosity gradient from one planar surface thereof to the other, said membrane impregnated with a dry chemistry reagent protocol specific for analysis of an analyte in the heterogeneous fluid sample. Said porous membrane further includes a sample receptive surface of sufficient density to selectively exclude substantial penetration of suspended or dissolved matter of the sample that might otherwise interfere with or mask the reaction of the analyte with the reagent protocol in the membrane, while allowing the remainder of the sample to be freely absorbed by said porosity gradient of said membrane. The receptive surface of the membrane is in substantially contiguous relationship with said lower surface of said wicking element.

It is an object of this invention to remedy the above and related deficiencies in the prior art. More specifically, it is the principal object of this invention to provide a dry reagent delivery system for clinical and immunoassay of biological fluids.

It is another object of this invention to provide a dry reagent delivery system which is effective in the analysis of biological fluids containing cells, particulate matter and macromolecules (hereinafter collectively referred to as "interferents")

without pretreatment (i.e. filtering or centrifugation) or dilution of the biological fluid or the chemical removal/neutralization of such interferents.

It is yet another object of this invention to provide a dry reagent delivery system suitable in the rapid and efficient analysis of whole blood for one or more analytes of interest. without prior separation of the serum from the cellular fraction.

It is still yet another object of this invention to provide a dry reagent delivery system suitable in the rapid and efficient analysis of urine for one or more analytes of interest, without prior concentration and/or chemical extraction of the analytes of interest from the urine sample.

It is an additional object of this invention to provide a dry reagent delivery system suitable in the rapid and efficient analysis of saliva for one or more analytes of interest.

It is yet an additional object of this invention to provide a dry reagent delivery system which is both self-contained and does not require any additional instrumentation or extensive training for performance of a diagnostic assay or interpretation of the result.

It is still yet an additional object of this invention to provide a dry reagent delivery system which is stable and retains its potency and activity until placed in use by contact with a biological fluid.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a perspective view of the inventive multi-layer test system.

Fig. 2 is a longitudinal cross-sectional view of the system of Fig. 1.

Fig. 3 is a top view of Fig. 1.

Fig. 4 is a bottom view thereof.

Fig. 5 is an exploded longitudinal cross-sectional view of the test system.

Fig. 6 is an exploded view of the inventive system.

Fig. 7 is a detailed view of the porous membrane element of the instant invention.

Fig. 8 is a view, similar to that of Fig. 7, however, showing a reagent deposited upon an upper planer surface of the membrane.

Fig. 9 is a detailed view showing the contiguous relationship between the wicking element and the porous membrane.

Fig. 10 is a partial breakaway view showing the sealing between upper and lower fluid impermeable layers bounding the wicking element and porous membrane and, as well, showing rectangular input and output apertures of said fluid impermeable layers. The pad of the system is not shown in Fig. 10.

Fig. 11 is a cross-sectional view taken along line 11-11 of Fig. 10 showing the fluid tight contact between opposing surfaces of said upper and lower fluid impermeable layers.

Fig. 12 is a partial breakaway view of the inventive system showing the fluid impermeable layers and an additional carrier element therefor.

Fig. 13 is a partial breakaway view of the inventive system in which a protective storage strip is provided to cover the pad element.

Fig. 14 shows the insertion of the inventive system into a device for reading the reaction of the analyte in the membrane

DETAILED DESCRIPTION OF THE INVENTION

Figs. 1 to 6 are illustrative of the internal structure of the reagent delivery system of this invention. A sample receptive surface (10) of a membrane (12) is essentially impermeable to cells and particulate matter. By way of contrast, the opposing surface 14 is relatively more porous. The matrix 16 of the membrane (12) (see Fig. 7) is fairly non-descript, except to note that its inherent void space has been reduced substantially from that originally existing by absorption of a conditioning agent, indicator, interactive reagents and flow control agents. Thus, the relative density of the original membrane has been effectively increased. The density or uniformity of the membrane containing the dry reagent system is believed to be critical to performance of assay. Also, the controlled drying and shielding of the indicator from photo-degradation are critical to both effective and consistent performance. The membrane uniformity is also critical to consistent performance of the test strip system of this invention.

A membrane which is suitable as a repository of the dry chemistry reagent system is, prior to conditioning, anisotropic, in that there exists a density gradient from one planar surface to the other. The density gradient is typically produced as an incident to its manufacture. More specifically, where the constituents of the membrane have been cast from a slurry, the particles of the slurry tend to settle (prior to and during the evaporation of the casting fluid) upon a supportive surface. Once the casting fluid has evaporated, the membrane forms a self-supporting film and can be separated from this supporting surface. The settling of particles of the slurry tends to create a more compact (dense) membrane surface contiguous to this supporting surface. The constituents from which the membrane can be prepared include both synthetic and naturally occurring materials, i.e., nylon, cellulose acetate, cellulose acetate-nitrate esters and mixtures thereof.

The membrane's physical characteristics (tensile strength, thickness, etc.) must be consistent with test strip manufacture; that is, it should have sufficient dimensional stability and integrity to permit sequential absorption and drying of the conditioning agent, the reagent protocol and/or indicator without loss of its physical strength. The physical attributes of the membrane (12) should also preferably provide sufficient durability and flexibility to adapt to automated processes for continuous manufacturing of test strips. The physical characteristics of the membrane should, in addition, be otherwise consistent with the absorption and retention of aqueous fluids in the contemplated environment of use.

The membrane (12) must also be relatively chemically inert; that is, essentially unreactive toward both the constituents of the chemistry reagent system and toward the constituents of a sample which is to be reacted with the reagent system within the membrane. It is, however, to be anticipated that certain of the inherent qualities of the membrane surface and/or its matrix may exhibit some affinity for a constituent of the reagent system and/or a constituent of the fluid sample. This natural attraction can, in certain instances, be used to advantage to immobilize a constituent of the reagent protocol and/or sample on or within a portion of the membrane and thereby effect a type of separation or anisotropic distribution of the constituents of the sample. This type of separation, based upon natural binding affinity of the membrane, can be used to advantage in both clinical chemistry assays and in immunoassay.

The membrane's optical properties should also enable effective observation/monitoring of the reaction manifesting indicator species. This requirement would, thus, contemplate that the membrane provide a background of sufficient contrast to permit observation of the indicator species at relatively low concentrations. Where the indicator is a fluorophor, the background fluorescence of

the membrane should be minimal or be essentially non-fluorescent at the monitored wavelength of interest.

Where the inherent characteristics of the membrane (12) are not conducive to effective monitoring of an indicator, it may be desirable to introduce a pigment into the dry chemistry reagent system. For example, certain of the membranes which may be potentially suitable for use in this invention can be colored or transparent. The introduction of pigment into the chemistry reagent system provides a suitable background against which to measure the indicator species.

Potential transparency of the membrane can, however, be used to advantage in both nephelometry and photo-fluorometric assays in which the presence or absence of analyte in the sample is manifest as a change in turbidity in the fluid phase. The dry chemistry reagent for such turbidimetric reaction systems will typically include a color, transparency or background modifying pigment which is absorbed into the membrane, preferably along with the conditioning reagent.

For the purpose of illustration, the fluid absorbent medium utilized in this reagent system can be any one of a variety of synthetic (preferably cast) membranes having a relatively dense surface 10 (pore size <0.5 microns) and a relative less dense surface 14 (pore size < 1.0 microns).

The order of absorption of the constituents of the dry chemistry reagent system into the membrane is generally dictated by considerations involving chemical compatibility and/or other factors relating to solubility in a common solvent. Since the o-tolidine solution is highly acidic, it is incompatible with enzymes of the reagent system and, thus, each are absorbed into the membrane separately.

Initially, the membrane is conditioned by treatment with a first solution containing protein, glucose, dextrin or dextrans, starch, polyethylene glycol (PEG), polyvinyl pyrolidone (PVP), or an equivalent. The purpose of such conditioning is two-fold: (a) to effectively reduce the void space within the matrix of the membrane and, (b) to assist or promote the absorption of the fluid fraction of the biological sample. In an alternative procedure for preparation of this reagent system, the conditioning agent can be combined with one or more of the interactive materials of the reagent system and concurrently absorbed into the membrane. Where the conditioning agent is combined with the interactive materials of the reagent composition, its absorption by the membrane will necessarily be preceded by absorption of the indicator molecule.

Where such conditioning of the membrane is effected independently of the interactive materials of the reagent system, the membrane is dried under controlled conditions, and then contacted with a second solution containing the indicator molecule (or the chemical precursor of the indicator molecule). The absorption of the indicator molecule (or its precursor) can be effected from either the less dense or more dense surface of the membrane, with preference being given to the less dense surface 14 with membrane 12. Following the absorption of the above solution, the membrane is once again dried under controlled conditions to preserve the uniformity of distribution of the non-fugitive constituents of this solution.

The membrane is then contacted with a third solution containing the balance (interactive materials) of the reagent system. The absorption of this solution is preferably effected by simply contacting the less dense surface of the membrane and this solution, and allowing the membrane to soak up the solution like a blotter. This third solution also contains what can be functionally described as a "flow control agent". This agent modulates the rate of speed/distribution of the fluid fraction of this sample throughout the matrix of the membrane. It is, thus, effective in the prevention

of the chromatographic separation of the reagents within the membrane matrix upon the addition of the fluid sample. Following addition of this third solution, the membrane is air dried for removal of excess fluid, lyophilized and shielded from light. The foregoing practices are necessary and appropriate for maintenance of uniformity of reagent distribution and protection against photo-degradation of the indicator.

The above described porous membrane may, accordingly, be understood as a bibulous film of essentially uniform composition in which the film's inherent fluid absorption and distribution characteristics have been modified by inhibition of a conditioning agent, an indicator, a fluid control agent and a reagent protocol into the matrix thereof. The effect of such absorption is to form an essentially uniform void volume within said matrix to enhance the uniformity of absorption and modulation of the rate of absorption of the fluid sample and of its interaction with the reagent protocol.

Once the reagent delivery system has been prepared as described above, the resultant membrane can be adapted to any one of several test strip configurations, specific for the analysis of whole blood, urine or saliva, in combination with the other components of the system described below.

The system generally employs said membrane (12) in a test strip system 18 intended for analysis of a biological fluid. This test strip configuration is intended for use in a protocol contemplating the application of the biological sample to a sample receptive pad 42, allowing for absorption of the fluid fraction of the sample thereinto and detection of an indicator molecule by inversion of the test strip and visually observing an indicator event upon the more porous surface (14) of the membrane. This test strip can, alternatively be inserted into a reflectance meter, nephlorometry (measuring turbidity), photometer, or fluorometer of the type illustrated in Fig. 14;

and, the reporter molecule measured and compared with a standard curve for the analyte of interest. The instrument will then report a value based upon its observation and comparison with the standard.

As may be noted in the views of Figs. 1 and 6, said lower surface 14 of membrane 12 is bonded to upper surface 20 of a lower fluid impermeable layer 22, the function of which is to provide structural support and protection against contamination of said membrane 12 as well to the system in general. As may be further noted in the views of Figs. 4 through 6, lower fluid impermeable layer 22 is provided with an aperture 24 through which is visible said lower surface 14 of membrane 12.

As may be noted in the views of Figs. 3, 5 and 6 there is also provided an upper fluid impermeable layer 26 having a top surface 28, a bottom surface 30 and an aperture 32 therewithin.

Further shown in the views of Figs. 1, 2, 5 and 6 is an essentially planar wicking element 34 having a top surface 36 and a bottom surface 38. Shown in lower contiguous relationship with wicking element 34 is a fluid impermeable barrier layer 40 having an aperture 41, which comprises an optional component of the system later described in fuller detail.

As may be seen in said figures, upper surface 36 of wicking element 34 is adhered to lower surface 30 of upper fluid impermeable layer 28.

Placed above said aperture 32 of upper fluid impermeable layer 28 is a conditioning pad 42, having an upper surface 44 and a lower surface 46. The pad is bonded to said surface 28 of said upper fluid impermeable layer 26 and comprises a porous compressible sponge-like material and comprises a means, further described below, for receipt of the heterogeneous fluid sample. As a result of the offset created

by aperture 32 between lower surface 46 of the said pad 42 and upper surface 36 of wicking element 34, a non-contacting relationship is maintained between pad 42 and wicking element 34, until pad 42 is deformably pressed against the aperture 32 so that it then touches wicking element 34 while pressure is applied.

It is to be appreciated that wicking element 34 facilitates the distribution of the fluid sample upon sample receptive surface 10 of the membrane 12 and may be also be used to effect a lateral transport of the sample from one portion of the membrane to another. Stated otherwise, the function of wicking element 34 in the structure of the instant multi-layer test system is twofold, namely, as an aid to the distribution of the sample over the sample receptive surface of the membrane 12 in the area defined by said aperture 32 and to effect absorption of excess sample fluid to thereby modulate the amount of fluid absorbed by the membrane 12, preventing such excess from inadvertent transfer to other surfaces, notably the optical surfaces within a monitoring instrument 58 of the type illustrated in Fig. 14. This limitation of the amount of the sample which is absorbed by the membrane produces a finite end point of the reaction which can be more easily monitored.

The wicking element 34 may be readily incorporated within an envelope defined by said upper and lower fluid impermeable layers 22 and 26 as is more, particularly, shown in the views of Figs. 10 and 11.

As may be noted in Fig. 6, a well 48 within wicking element 34 is preferably positioned to coincide with said aperture 32 of impermeable layer 26 of the enclosing envelope. Upon application of a whole blood sample to the pad 42, the blood is essentially uniformly drawn by the wicking element 34 from its point of application to the surface of the membrane. By facilitating the distribution of the sample in this manner, the reaction of the analyte with the dry chemistry reagent system proceeds more uniformly, thus avoiding unevenness in color or indicator development. The

presence of wicking element 34 is also desirable where it is used to laterally transport the whole blood sample from its point of application on the test strip to the situs of reaction, or where it contains reagents to pretreat or condition the sample prior to its absorption into the membrane 12. Further, if, is shown in Fig. 9, a contiguous layer of the wicking element 34 is used upon membrane 12, the flow through the membrane may be modulated by changes in the concentration of flow control components impregnated in the wicking element. Within an increase in the concentration in these components, the flow rate across the membrane 12 may be reduced and the usable range of measurement of the test system preserved, thus compensating for the presence of a contiguous wicking element.

With further reference to said pad 42, this element will preferably comprise a porous compressible sponge-like material, i.e., fiberglass, neoprene, or a cellulose material. Such pad 42 can also be treated with a saline solution containing any one or combination of components to enhance the ability of the dry chemistry reagent system impregnated within membrane 12. The function of the compressible pad 42 is particularly evident in tests of whole blood samples for the detection of high density lipoproteins (HDL).

Since the body's ability to absorb and/or transport cholesterol, is dependent upon the density (molecular weight) of the lipoproteins (which are polymeric compounds associated with the steroid), the importance of measuring and quantifying HDL, from the total cholesterol, and/or from low density lipoproteins (hereinafter "LDL") can be of paramount importance. In Example 1A below, the sample-conditioning pad (42) is pretreated with the reagents which precipitate the low density lipoproteins. After waiting for a brief period for this interaction of preconditioning to be completed, the conditioned sample is expressed onto the sample receptive surface of a cholesterol test strip which has been optimized for higher sensitivity to cholesterol. Since the precipitated LDL are trapped along with a portion of the

cellular fraction of the sample within the preconditioning pad or on the relatively dense surface of the membrane of the test strip, primarily the HDL reaches the dry chemistry reagent system within the test strip, thereby initiating a color reaction which is manifest on the opposing surface of the test strip. The indicator produced as a result of this reaction can be observed or measured quantitatively and, thusly, correlated with the amount of HDL present in the sample.

With reference to Figs. 4 and 5 it is noted that lower aperture 24 functions to expose the more porous lower surface 14 of membrane 12 thereby permitting monitoring of a reaction product which is indicative of the presence or absence of the analyte of interest. This ability to observe/measure such reaction product without physical removal of sample residue from the sample receptor surface of the membrane 12, or to optically mask such residue with a separate blocking layer in the nature of an optical screen, are a particularly advantageous aspects of the present invention.

Fig. 8 is illustrative of an embodiment of the system of the invention in which a component of the reagent protocol of membrane 12 is deposited upon the sample receptive surface 10 as a thin layer or coating 50. Such a separation of the constituents of the membrane reagent system from one another in the dry stage is only a temporary condition. That is, upon application of the fluid sample to this coating, the analyte of interest will displace the layered component of the reagent system so that it is freely absorbed into the membrane 12 to initiate a discernible reaction which is indicative of the presence or absence of the analyte of interest. It is to be understood that coating 50 may, in a given protocol, be adhered to the lower surface 14 of the membrane 12 to assist in the viewing of the reaction product through aperture 24. See Fig. 4.

Fig. 8 also illustrates the adaptation of the test strip of this invention to a displacement immunoassay of the type described in Liotta U.S. Patent 4,446.232, which is hereby incorporated by reference in its entirety. In the device exemplified in Fig. 8, the sample receptive surface of the membrane is coated with an enzyme labeled antigen or antibody (hereinafter "enzyme labeled conjugate"). The method of application of the coating to the sample receptive surface insures against penetration of the coating material into the matrix of the membrane. The balance of the immunochemistry reagent system, notably, a chromogenic or fluorogenic substrate for the enzyme, is incorporated into the conditioned membrane, so as to preserve its physical isolation from the surface coating. The contact of the sample with the coating on the surface of the membrane, results in displacement of enzyme labeled material. The displacement of the enzyme labeled conjugate is based upon the dynamic equilibrium which is caused by the presence of an analyte in the sample and the competition with the conjugate for binding to an analyte mimic in the surface coating.

The displaced enzyme labeled conjugate, along with a portion of the fluid fraction of the sample, is absorbed in the matrix of the membrane. The enzyme portion of this conjugate interacts with a substrate specific for the enzyme and thereby produces a discernible change in color or fluorescence which is indicative of the analyte of interest. This change can be observed visually (in the case of a color change) or by instrumentation designed for that purpose.

With respect to barrier layer 40 (see Figs. 1 through 6), this element represents an additional/optional aspect of the invention, the function of which is to control or focus the fluid output of wicking layer 34 and, thereby, obtain a more precisely defined analytical test site within the membrane 12. In other words, the effect of barrier layer 40 is to effectively confine the fluid sample to wicking layer 34 with the

exception of area 41 of the barrier layer 40 which operates to create an analytical test site within the membrane.

It is to be appreciated that in an embodiment of the instant invention in which it is appropriate to have a plurality test sites within the membrane, a plurality of apertures 41 within the barrier layer 40 may be provided.

With reference to Fig. 10 there is therein illustrated the inventive system in a configuration which more nearly approximates the external geometry which the system would exhibit as a production product. That is, as may be noted in the views of Fig. 10 , the entire structure, including upper and lower fluid impermeable layers 122 and 126, membrane 12 and wicking layer 34, are very flat such that the vertical dimension of each element is very small. Resultingly, each layer of the manufactured structure shown in Figs. 10 and 11 exhibits the general appearance of a flat strip of paper. Also, in the embodiment of Figs. 10 and 11, aperture 132 within upper fluid impermeable layer 126 is shown in a rectangular configuration, as opposed to the circular configuration of aperture 32 of the embodiment of Figs. 1 through 6. It is to be understood that either geometry may be employed in either embodiment of the invention.

As may be seen in the transverse cross-sectional view of Fig. 11, a substantial area of interface exists between upper and lower fluid impermeable layers 126 and 122 to provide sufficient environmental protection to the membrane 12 and wicking element 34. For purposes of clarity the pad of the system is not shown in the views of Figs. 10 and 11.

In the view of Fig. 12 is shown a further embodiment in the instant invention in which the upper fluid impermeable layer 126 is provided with a longitudinal slot 54 which functions as an air bleed within the envelope defined by layers 122 and 126 to

permit the escape of air that may become trapped in the envelope and/or to allow for escape of gases from the reaction of the reagent protocol. Also shown in the embodiment of Fig. 12 is protective film 52 which may be placed upon lower fluid impermeable surface 122 to provide further environmental protection to the system. A system of the type of Fig.12 is therefore suitable for insertion in a fluid sample, i.e., a dipstick type sampling.

In the embodiment of Fig. 13 a protective film 56 is shown upon the top of the structure, that is, in contact in relationship with pad 42. It is to be further appreciated that film 56 may, additionally, be provided on bottom of the embodiment of Fig. 12 to provide a further degree of environmental protection to both sides of the inventive system. This confinement of the sample within the test strip provides significant protection of the clinician from inadvertent contact with an infectious specimen; and, also prevents unintended transfer of sample residue to surfaces of an analytical device (i.e. fluorometer) which can be used to monitor the concentration of an indicator produced within the membrane. With respect to this latter point, the test strip is compatible for use in a variety of environments, including use with a monitor of the type illustrated in Fig. 14.

In Fig. 14 is shown a monitor 58 the function of which is to receive a test strip of the type shown in Figs. 10 and 11 within the door 60 thereof. This is accomplished by orienting the test strip in a manner to align the bottom aperture 24 within the monitor 58, i.e., the aperture 24 within lower surface 22 of the system is photo-optical elements (not shown) of the monitor 58. After a requisite interval, the monitor has had an opportunity to measure the level of indicator in the test strip. Thereafter test strip is removed from the monitor and discarded.

A test strip system for triglycerides (triacylglycerols) can be prepared by absorption into the pad 42 and/or the a conditioned membrane 12, of surfactant,

lipase, adenosine triphosphate (ATP), glycerol kinase and L-alpha-glycerol phosphate oxidase, and a triarylimidazole leuce dye. In brief, the surfactant aids in dissociation of the lipoprotein complex so that the lipase can react with the triglycerides to form glycerol and fatty acids. The glycerol is then phosphorylated with the adenosine triphosphate in the presence of the glycerol kinase enzyme. The L-alpha-glycerol phosphate thus produced is then oxidized by the L-alpha-glycerol phosphate oxidase to dihydroxy acetone phosphate and hydrogen peroxide. The hydrogen peroxide oxidizes the leuco dye, producing a colored indicator which has a peak absorption at 640 nm. Additional details relating to this specific reagent system appear in the Spayd publication, i.e., Spayd, Richard W. et al, "Multilayer Film Elements for Clinical Analysis," Clinical Chemistry, Vol. 24, No. 8, pp. 1343-1350 (1978).

An alternative and preferred chemistry reagent system for triglyceride analysis can be prepared by absorption, into the pad 42 and/or the conditioned membrane 12, of lipase, glycerol dehydrogenase, p-iodonitrotetrozolium violet (INT) and diasphorase. The serum triglycerides initially interact with the chemistry reagent system and are hydrolyzed to free glycerol and fatty acids. The free glycerol is now converted to the dihydroxyacetone by glycerol dihydrogenase, in the presence of NAD. Simultaneous with such conversion, INT (colorless) is reduced by diaphorase, in the presence of NADH, to red dye (maximum gamma = 500 nm). The change in absorbence of the test strip at 500 nm is directly proportional to the concentration of serum triglycerides.

A test strip for determination of total cholesterol in serum can be prepared by absorption, into the conditioned membrane (12), of cholesterol ester hydrolase, cholesterol oxidase, a leuco dye and peroxidase. Upon application of a whole blood sample to the sample receptive surface 10, of the membrane 12, the serum is absorbed into the membrane, thereby initiating conversion of the cholesterol esters to

cholesterol, the oxidation of the cholesterol is accomplished by the cholesterol oxidase enzyme, thereby liberating peroxide. The peroxide and leuco dye then interact in the presence of peroxidase to form a highly colored indicator which can be monitored either visually or through the use of instrumentation. Additional details relating to this specific reagent system appear in the literature. See Dappen, G.N., et al., Clin. Chem., Vol. 28, No 5 (1982), 1159.

A test system for urea can be prepared by absorption, into a conditioned membrane, of appropriate concentrations of urease, buffer, and an indicator sensitive to changes in pH. When a whole blood sample is brought in contact with the sample receptive surface of the membrane, the serum is taken up by the membrane. The urea present in the serum is digested by the urease enzyme thereby liberating ammonia in solution. The ammonia can then react with a suitable indicator (i.e., a protonated merocyanine dye). The pH of the membrane is buffered to about 8.0 to keep the equilibrium concentration of the ammonia relatively low. The indicator is monitored at 520 nm. Additional details of this specific reagent system are described in the open literature, see example Spayd, R.W., above referenced.

A test system for alpha-amylase can be prepared by absorption, into a conditioned membrane, of appropriate concentrations of a derivatized substrate (i.e., starch) and buffer. When the whole blood sample is applied to the sample receptive surface, 12 of the test strip, (18), the serum is absorbed in the membrane, thus initiating digestion of the derivatized substrate by the alpha-amylase in the sample. This digestion of the substrate releases a chromophore or fluorophore which can be monitored in accordance with accepted techniques and readily available equipment. Additional details for this specific reagent system also appear in the Spayd publication, previously referenced herein.

A test system for bilirubin can be prepared by absorption, into a conditioned membrane, of appropriate concentrations of certain cationic polymers (i.e., polymeric quaternary salts) and phosphate buffer (pH approximately 7.4). When a whole blood sample is applied to the sample receptive surface of the test strip, the serum is absorbed into the membrane, thereby initiating interaction of the bilirubin and the cationic polymer. Such interaction results in a shift in the maximum absorption of the bilirubin from 440 to 460 nm with an accompanying substantial increase in absorption at the new peak. Additional details relating to this specific reagent system also appear in the previous referenced Spayd publication.

A test strip for creatinine can be prepared by absorption into a conditioned membrane of appropriate concentrations of creatinine iminohydrolase and an ammonia indicator (i.e., bromphenol blue). Upon application of a whole blood sample to the sample receptive surface of the test strip, the serum is absorbed into the membrane, thereby initiating interaction of the creatinine and the enzyme, creatinine amino hydrolase, resulting in the liberation of ammonia. The ammonia thereupon reacts with the indicator and the color development monitored visually or with conventional instrumentation. Additional details relating to the specific reagent appear in the literature, see for example, Toffaletti, J., et al., Clin. Chem., Vol. 29, No. 4 (1983), 684.

Examples of the inventive test system follow:

EXAMPLE 1

The dry chemistry reagent system of this invention can be further adapted to a total cholesterol test strip incorporating the sample conditioning pad. The sample conditioning pad 12. The sample conditioning pad is preferably pre-positioned over the aperture 32 of the wicking layer 34. In one of the commercial embodiments of this device, the sample conditioning membrane 12 is physically integrated/attached to the envelope 22/28 housing the membrane. The whole blood sample is applied to the sample conditioning pad which promotes the release of the cholesterol from the cholesterol binding protein of the sample. This sample conditioning pad also promotes a degree of physical separation of the cellular matter from the serum fraction of the sample; however, such physical separation is not believed to be a prerequisite to its effectiveness in the release of cholesterol from the binding protein, nor the ability to express the conditioned sample onto the sample receptive surface 10 of the membrane 12.

The dry chemistry reagent delivery system is prepared in accordance with the procedures described herein from the following materials:

(a) Membrane

millipore MF (mixed cellulose acetate-nitrate, and cellulose acetate)
density 4.9 - 6.4 mg/cm^2
porosity 0.01 - 0.45 (sample receptive surface)

(The porosity range is indicative of the differences in pore size from the more dense surface to the less dense surface.

(b) Indicator - 1% (w/v) aqueous solution

deionized water
o-tolidine hydrochloride

(c) Cholesterol specific reagent cocktail

cholesterol esterase	40 U/ml
cholesterol oxidase	40 U/ml
peroxidase	148 U/ml

0.1 M Citrate Buffer

Stabilizer for enzyme - albumin 0.2% (w/v)
Conditioning and flow control agent - polyvinyl pyrolidone 3% (w/v)

(d) A sample conditioning pad is prepared by treatment of a compressible sample adsorbent material with an aqueous salt solution (saline) containing a sample conditioning agent. Where the sample conditioning pad is a fiberglass mat (retention rating of 0.53-3μm), the conditioning agents TRITON X-100, Sodium Chloride and Sodium Nitrate Alternative embodiments of conditioning pad include sponge like materials consisting of cellulose or neoprene which contain 100U Thrombin and physiological salts.

(e) Wicking element. A chromatographic type filter paper, of a type commercially available from Alston or Schleiker & Solule, is used with such paper readily wets and aids in the uniform spreading of the expressed sample throughout the surface of the membrane. Alternatively, a liner cloth of untreated cotton, or cotton polyester-blend, having significant density to provide uniform flow, e.g., 180 to 220 threads per inch, may be utilized.

The sample conditioning pad is positioned over the aperture of the test strip which exposes the sample receptive surface of the test strip membrane. The whole blood sample is applied to the sample conditioning pad in the same manner as contemplated for use of the other test strips of this invention. After the sample is taken up by this pad, and the interaction of the pad and the sample allowed to go to completion (generally within 30 to 60 seconds), the pad is manually compressed, thereby expressing the conditioned sample onto the wicking element which affords the spreading function (above described) and therefrom to the sample receptive surface of the membrane. The fluid fraction of the expressed sample is readily absorbed by the membrane, interacts therewith and produces a color reaction which is readily correlated with color chart/index which correlates the color with a value or level of cholesterol in the sample.

EXAMPLE 1A

The system of Example 1 above may be adapted to use and a high density concentration test strip by doubling the strength of the reagent cocktail of Paragraph (d) above is further providing sensitivity to cholesterol.

Further the conditioning pad of Paragraph (d) above is further provided with phosphotungstic acid or dextran sulfate to precipate out the low density lipoprotein (LDL) of the sample.

EXAMPLE 2

The following immunoassays adapt the technology of this invention to some of the immunoassays which have traditionally been performed in solution, in the classic heterogeneous format or in a multi-laminate solid phase format.

A test strip is prepared by initially coating a thin film containing a quantitative amount of peroxidase labeled beta-human chorionic gonadotropin hormone (beta-HCG conjugate) onto the sample receptive surface of the membrane of the same type used in Example 1. The beta-HCG conjugate is effectively immobilized in this coating by immunochemical binding of the antibody portion of the conjugate to a hapten mimic which is also present in the coating. Precautions are taken to insure that the conjugate is not also absorbed into the membrane matrix. Following such surface coating, the balance of this reagent system is absorbed into the membrane by absorption from the relatively porous side. The sequence of absorption is the indicator, o-tolidine (in an acidified vehicle) is absorbed first, followed by a second solution containing glucose oxidase, citrate buffer glucose and conditioning agent.

A freshly voided same of urine from a pregnant woman is collected in the early morning. This sample is then applied to the pad by simply dipping the test strip into the urine specimen. The beta-HCG in the urine specimen is, thru the wicking element brought into contact with the coating on the sample receptor surface of the test strip and a portion of the beta HCG enzyme conjugate is displaced and binds to the analyte. This displacement of the conjugate by the analyte allows the conjugate to be absorbed into the membrane, where the enzyme portion thereof reacts with its corresponding substrate (H_20_2), ultimately resulting in oxidation of the o-tolidine (and its conversion to a colored indicator). The development of color is, thus, indicative of the presence of analyte in the sample.

EXAMPLE 3

A test system suitable for use in a competitive enzyme immunoassay is prepared by initial absorption of a substrate/color forming composition of Example 1 into the conditioned membrane. Also absorbed into the membrane is an enzyme (peroxidase) labeled antigen (i.e. beta-HCG). A specific quantitative amount of human beta-HCG antibody is coated onto the sample receptive surface of the membrane. A urine sample is then applied to the sample receptive surface of the membrane and the beta-HCG antibody on the surface reacts with the analyte in the sample. The immunochemical interaction of the analyte with the beta-HCG antibody impedes the absorption of the antibody into the matrix of the membrane. The relative concentration of analyte thus modulates the intensity of development of the indicator; the higher the concentration, the less intense color development. The extent of color development can be monitored visually or through the use of instrumentation.

Alternative indicator systems include fluorescent label conjugates (FIA) or radioisotope labeled conjugates (RIA). In each of these alternate assay formats, indicator concentrations would be monitored with instrumentation.

EXAMPLE 4

An immunoassay is adapted to the conditioned membrane assay format of this invention by imbibing beta-HCG labeled colloidal gold particles and/or should permanently labeled HC antibody or colored polystyrene latex. An aliquot of sample (i.e. urine) is then applied to the sample receptive pad and allowed to migrate thru the wick to the membrane. The presence of antigen in the sample is manifested by the migration of color between the membrane which can be trapped by the use of additional suitable antibodies preimpregnated into the membrane surface for visual observations.

The foregoing Description and Examples have been provided as illustrative of a representative number of the preferred embodiment of this invention. It is not the intent of such Description and Examples to delineate the scope of this invention, which has been reserved to the claims that are set forth hereinafter.

THE CLAIMS

Having thus described my invention what I claim as new, useful and non-obvious and, accordingly, secure by Letters Patent of the United States is:

1. A dry chemistry reagent system suitable for detection of an analyte in a heterogeneous fluid sample, the system comprising:

(a) a pad for pre-conditioning said sample, the pad comprising a porous deformable compressible sponge-like material, said pad comprising means for receipt of the fluid sample, said pad impregnated with means to (i) facilitate binding therewithin of suspended or dissolved matter of said sample that might otherwise interfere with or mask detection of said analyte or (ii) pre-treat said sample to facilitate a further reaction, said paid having an upper and lower surface;

(b) an essentially planar wicking element having an upper and lower surface, said upper surface in proximal non-contacting relationship to said lower surface of said pad, said wicking element comprising means for facilitating transport and uniform spreading of the said fluid sample;

(c) an upper fluid impermeable layer situated between said pad and said wicking layer, said upper impermeable layer having an aperture therein for fluid communication between said lower surface of said pad and said upper surface of said wicking element:

(c) an essentially planar porous membrane having a porosity gradient from one planar surface thereof to the other, said membrane

impregnated with a dry chemistry reagent protocol specific for analysis of an analyte in the heterogeneous fluid sample, said membrane further having a sample receptive surface of sufficient density to selectively exclude substantial penetration of suspended or dissolved matter of the sample that might otherwise interfere with or mask the reaction of the analyte with the reagent protocol in the membrane, while allowing the remainder of the sample to be freely absorbed by said porosity gradient of said membrane, said receptive surface of said membrane in substantially contiguous relationship with said lower surface of said wicking element and said other surface of the membrane comprising a lower surface thereof; and

(e) a lower fluid impermeable layer situated beneath said lower surface of said membrane.

2. The system as recited in Claim 1, in which said porous membrane comprises:

a bibulous film having an essentially uniform void volume within a matrix in which said film's inherent fluid absorption and distribution characteristics have been modified by imbibing a conditioning agent, an indicator, a flow control agent and said reagent protocol into its matrix thereof, the effect of such absorption being to enhance the uniformity of internal structure of said film and its uniformity of absorption, and to modulate the rate of absorption of the fluid sample and its interaction with the reagent protocol.

3. The system as recited in Claim 2, in which said bibulous film is conditioned with an absorption-effective amount of conditioning agent selected from the group consisting of albumin, polyvinyl pyrolidone, polyethylene glycol,

carbohydrates, carboxymethyl cellulose, methyl cellulose, glycerine and polyoxyethylene ethers.

4. The system as recited in Claim 2, in which said bibulous film is selected from the group consisting of a cellulose acetate, cellulose nitrate ester, nylon and polysulfone.

5. The system of Claim 2, in which the reagent protocol is specified for detection of cholesterol, selectably including the detection of high density lipoproteins.

6. The system of Claim 2, in which the reagent protocol is specific for detection of urea.

7. The system of Claim 2, in which the reagent protocol is specific for detection of an antigen or antibody.

8. The system of Claim 7, in which the reagent protocol includes an antigen or an antibody which is conjugated to a mimic of an analyte.

9. The system of Claim 7, in which the reagent protocol includes an immunoglobulin binding site or a mimic thereof.

10. The system of Claim 7, in which the reagent protocol is specific for detection of hormones, pharmaceutical compounds, drugs of abuse, or proteinaceous materials capable of evoking an immune response.

11. The system of Claim 2, in which a portion of the reagent protocol is deposited upon one of the planar surfaces of the bibulous film to be retained thereon without substantial penetration into the matrix of the bibulous film.

12. The system as recited in Claim 11, in which said lower fluid impermeable layer includes therein an aperture to permit viewing of said lower surface of said membrane.

13. The system as recited in Claim 2, in which said wicking element comprises means for further pre-conditioning said sample.

14. The system as recited in Claim 13, in which said system includes a plurality of said pads in fluid communication with said wicking element.

15. The system as recited in Claim 1, further comprising:

a fluid impermeable barrier layer situated between said wicking element and said sample receptive surface of said porous membrane for maintaining said wicking element out of fluid contact with said receptive surface, said barrier layer having at least one aperture for confinement of a pool of sample on the receptive surface of the porous membrane, the size and shape of said aperture permitting flow of the heterogeneous fluid sample from the wicking element to the sample receptive surface of the membrane and roughly corresponding to an analysis site.

16. The system as recited in Claim 5, in which the heterogeneous fluid sample is a whole blood and the wicking element is transportive of the cholesterol of the blood sample to be analyzed in an unbound state of said cholesterol.

17. The system as recited in Claim 16, in which said lower fluid impermeable layer includes therein an aperture to permit viewing of said lower surface of said membrane.

18. The system as recited in Claim 15, in which said porous membrane comprises:

a bibulous film having an essentially uniform void volume within a matrix in which said film's inherent fluid absorption and distribution characteristics have been modified by imbibing a conditioning agent, an indicator, a flow control agent and said reagent protocol into its matrix thereof, the effect of such absorption being to enhance the uniformity of internal structure of said film and its uniformity of absorption, and to modulate the rate of absorption of the fluid sample and its interaction with the reagent protocol.

19. The system as recited in Claim 18, in which the heterogeneous fluid sample is a whole blood and the wicking element is transportive of the cholesterol of the blood sample to be analyzed in an unbound state of said cholesterol.

20. The system as recited in Claim 13 in which said pad is impregnated with a reagent to facilitate binding of low density lipoproteins to said pad, permitting the fluid sample inclusive of high density lipoproteins of cholesterol to be expressed into the wicking layer for further assay.

21. The system as recited in Claim 16 in which said pad is impregnated with a reagent to facilitate binding of low density lipoproteins to said pad, permitting high density lipoproteins of cholesterol to be expressed into the wicking layer for further assay.

22. The system as recited in Claim 13 in which said pad is impregnated with a coagulation agent to facilitate separation of blood serum from red blood cells thereof.

23. The system as recited in Claim 16 in which said pad is impregnated with a coagulation agent to facilitate separation of blood serum from red blood cells thereof.

FIG. 1.

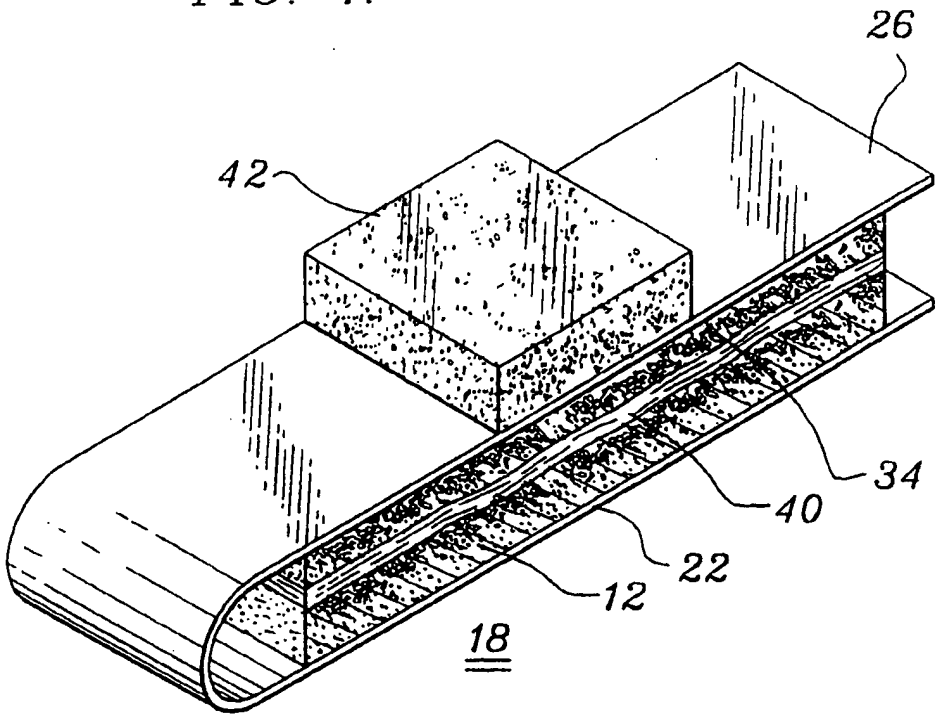


FIG. 2.

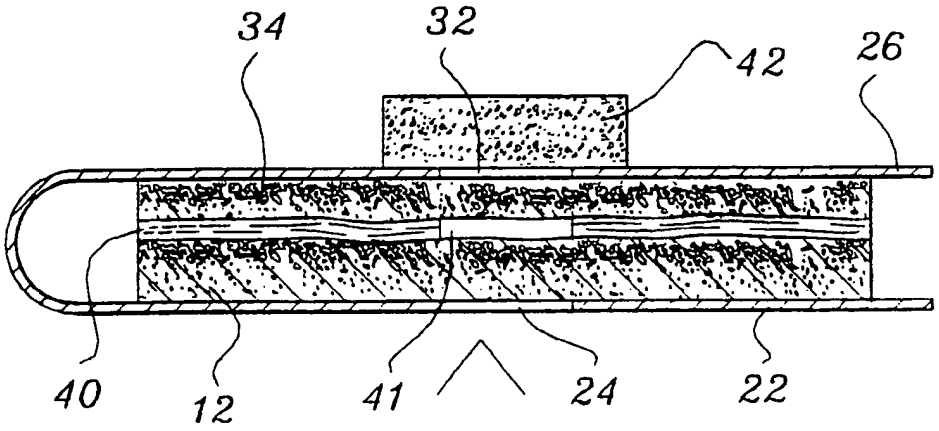


FIG. 3.

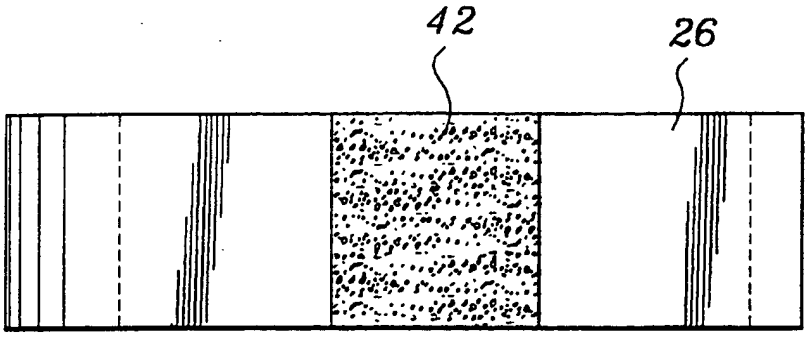


FIG. 4.

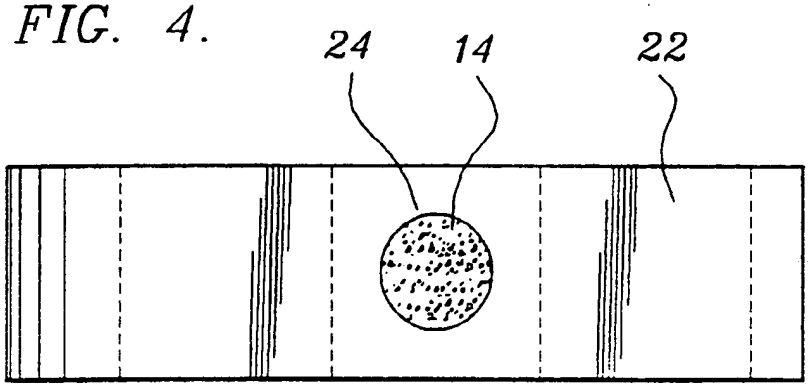


FIG. 5.

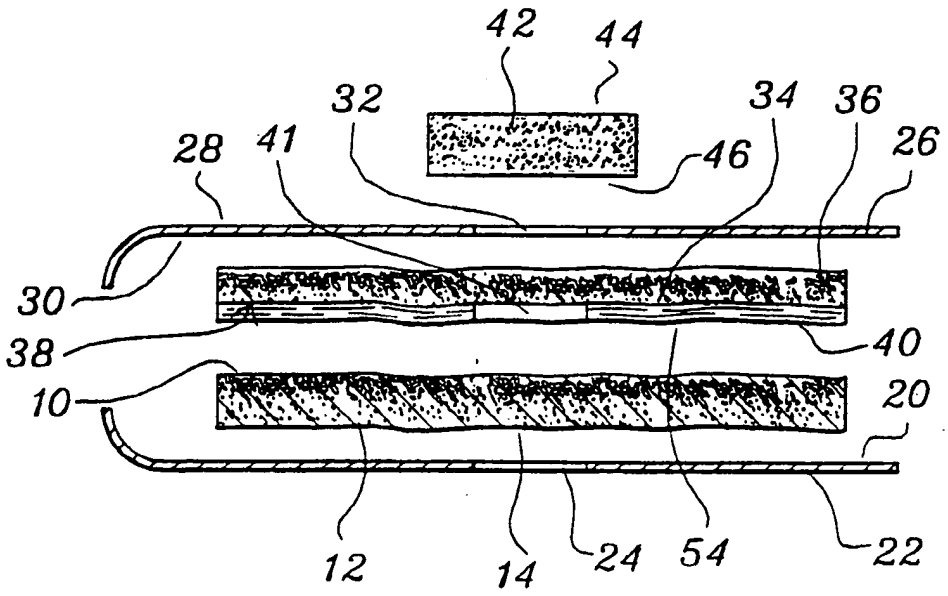
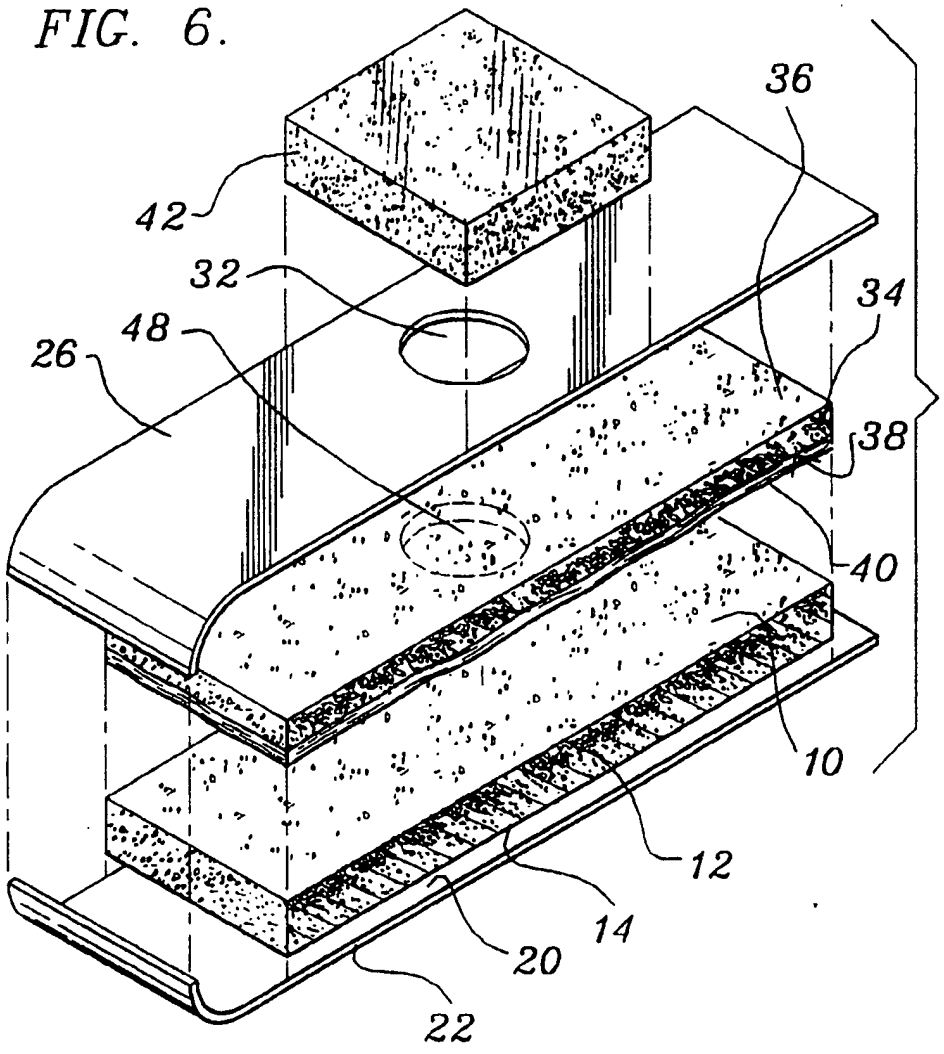


FIG. 6.



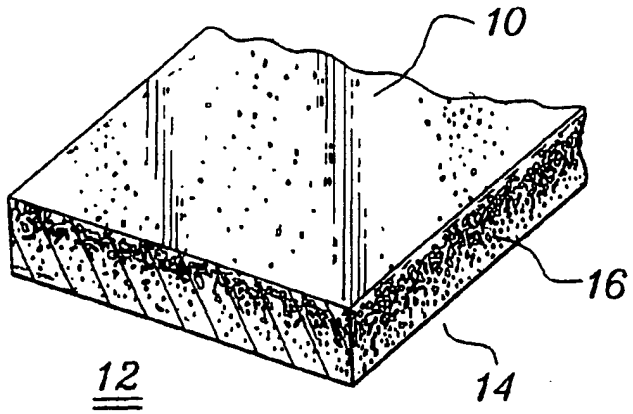
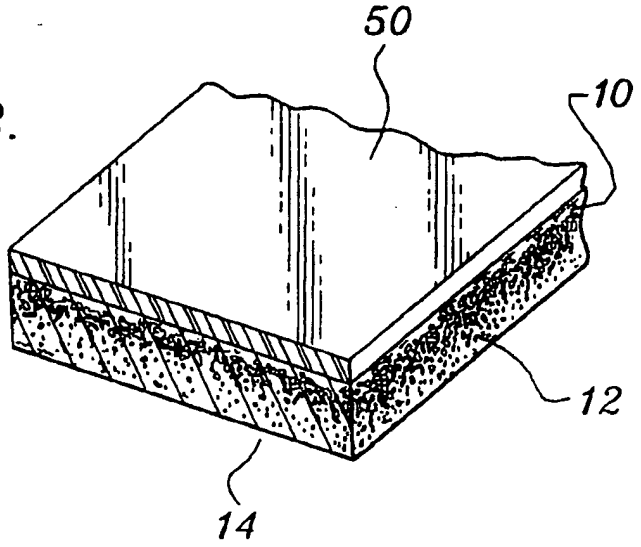


FIG. 7.

FIG. 8.



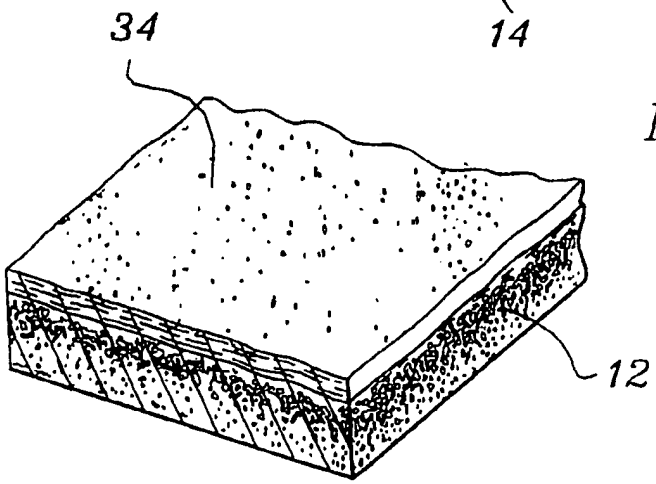


FIG. 9.

FIG. 10.

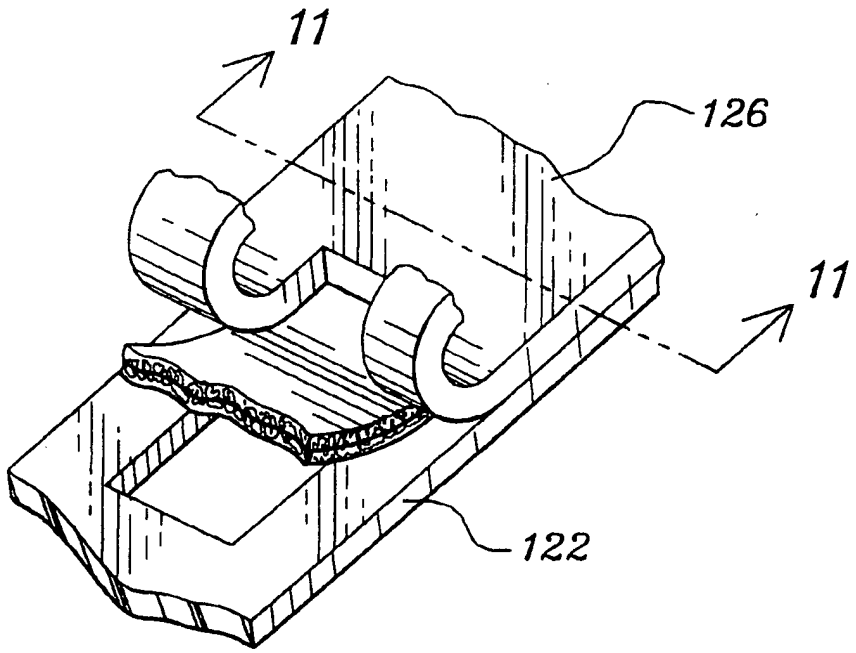


FIG. 11.

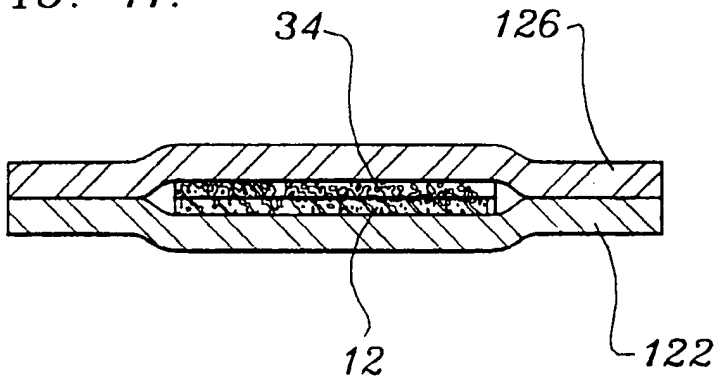


FIG. 12.

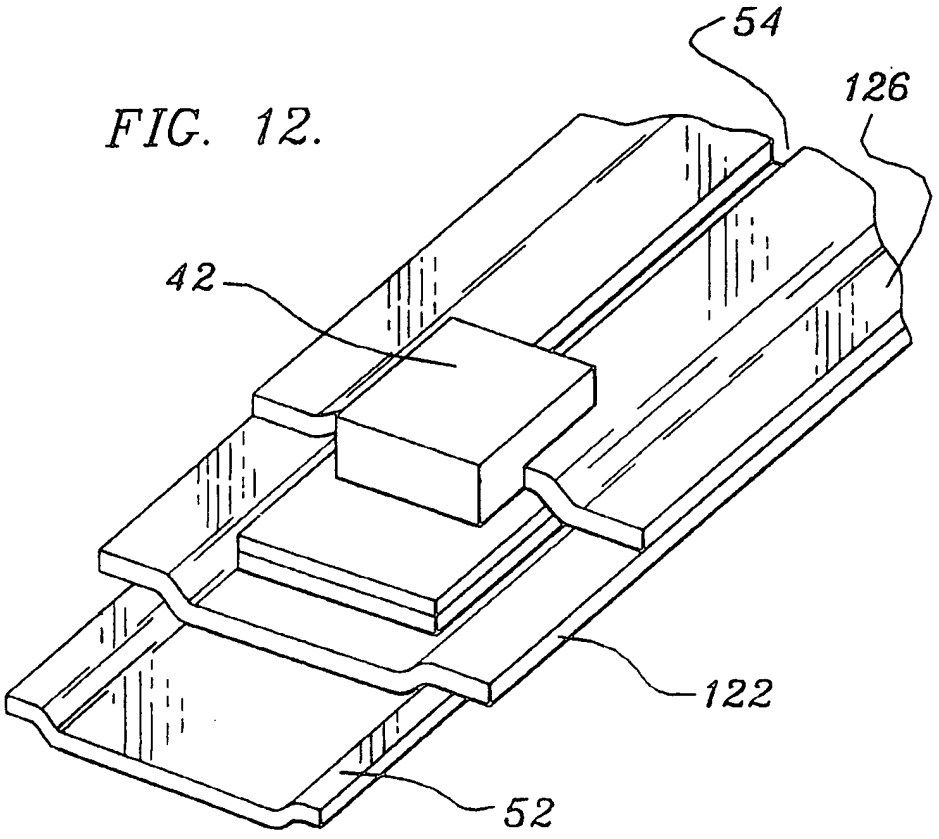


FIG. 13.

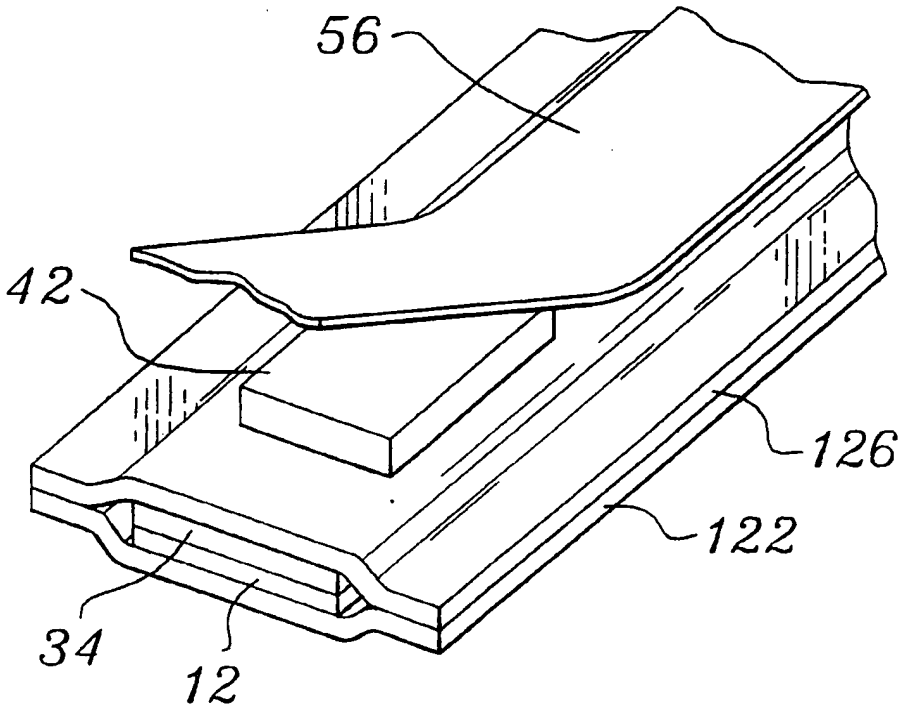
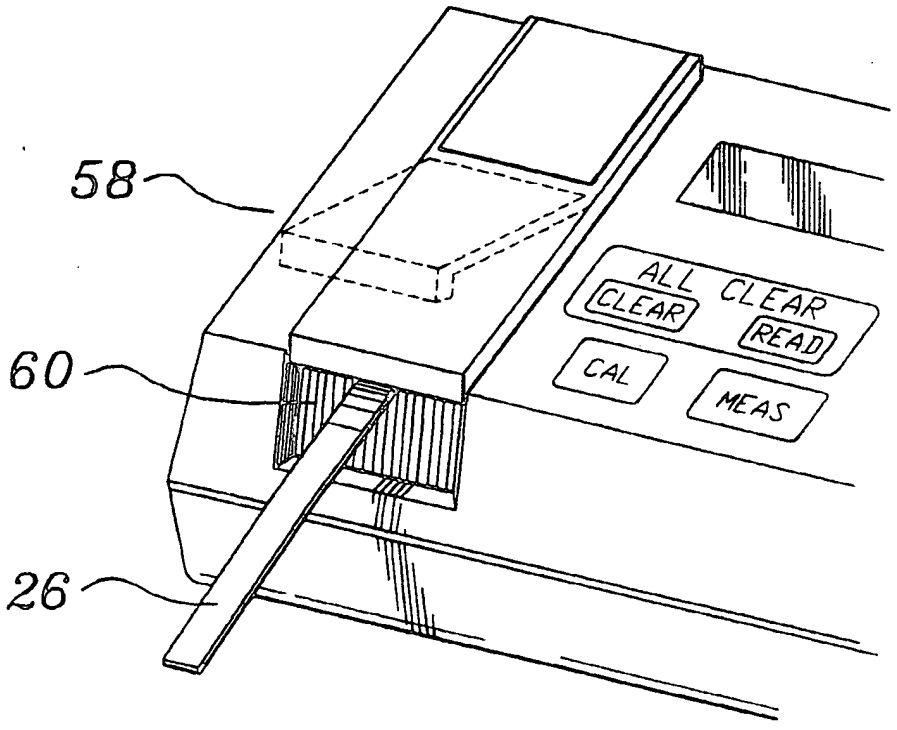


FIG. 14.



INTERNATIONAL SEARCH REPORT

Ir ational application No.
PCT/US93/11482

A. CLASSIFICATION OF SUBJECT MATTER
IPC(5) :G01N 33/543
US CL :436/518
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 436/66,69,169,170,174.177,514,518,528,530,531,805,810,815-818; 435/805,970; 422/55-58

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

None

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

None

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,256,693 (Kondo et al), 17 March 1981, see entire document.	1-23
Y	US, A, 4,987,085 (Allen et al), 22 January 1991, see entire document.	1-4,6-19, 22,23
Y	US, A, 4,774,192 (Terminiello et al), 27 September 1988, see entire document.	1-23
Y	US, A, 4,994,238 (Daffern et al) 19 February 1991, see entire document.	12,15,17-19
Y	US, A, 5,135,716 (Thakore) 04 August 1992, see entire document.	1-22

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
05 January 1994

Date of mailing of the international search report
07 FEB 1994

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Summary

Document	Pages	Printed	Missed
US005747274	36	36	0
WO009412879	52	52	0
Total (2)	88	88	0